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Toxic Stress Exhibited by Juveniles of *Clariasgariepius* Exposed Toacute Concentrations of Vestaline (Pendimethalin) Herbicide

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ABSTRACT

The studyAcute effects of vestaline (pendimethalin) herbicide exposed to juveniles of African catfish (*Clarias gariepinus*) was carried out to determine the toxicity of the herbicide using static bioassay. The study was designed with emphasis on the effects of Vestaline (Pendimethalin) on the behavioural and mortality rate of *Clarias gariepinus* juveniles. Also to study the effects of Vestaline (Pendimethalin) herbicideonphysico-chemical parameters of exposed water. The acute toxicity was carried out using 180 healthy fish of mean weight 27.97±0.03g which were divided into six treatments with each treatment having ten fish and the setup was in triplicate. The fish were exposed to 33,82.5,115.5,148.5,181.5mg/l concentrations of Vestaline (Pendimethalin) for 96 hours with control (0mg/l) also setup. The behavioural responses observed during the acute toxicity were; aggression, mucus secretion, erratic swimming, air gulping and discoloration. Data obtained were subjected to analysis of variance (ANOVA) and Regression at $p< 0.05$using Minitab 17.The results showed that all the behavioural responses were dose dependant. Also there was no mortality in the control (0.00mg/l) setup while mortality increased with increased the control (0.00) setup, while mortality increased with increase in concentration. The LC50 value of Vestaline was determined to be 113.56mg/l. Results of the physico-chemical parameters of water exposed to acute concentrations decreased significantly ($p<0.05$) with increase in concentration for dissolved oxygen and increased in pH, Total dissolved solids (TDS) and Electrical conductivity(EC) while Temperature showed no significant difference ($p>0.05$). Vestaline (Pendimethalin) herbicide from this study is toxic on *Clarias gariepinus* juveniles and should be used with care.

INTRODUCTION

Humans rely on surface freshwater ecosystems for many goods and services (drinking water, recreational facilities), which places these systems at the center of a web of ecological, economic and political interests (Wilson*et al*., 2003). They are also being subjected to increasing pressure resulting from anthropogenic activities, including contamination by a variety of mineral and organic pollutants. Most of the organic pollutants are herbicides (Kreuger, 1998), which are used not only in agriculture but also for many other purposes (ranging from domestic, use in gardens and maintaining power plants). These herbicides can enter aquatic ecosystems as a result of terrestrial runoff, and to a lesser extent, of direct application and aerial spraying (Carter, 2000).

Herbicides are the chemicals which are employed to kill or control vegetation. Common salt, ash etc. have been used for centuries to control weeds, but selective control of weeds in agriculture was first conceived in 1896 in France, when Bordeaux mixture sprayed on grapevines for protecting it from downy mildew damaged certain broadleaf weeds. Herbicide development in the 1980s was marked by the introduction of selective post-emergence treatments in major crops such as sulfonylureas, imidazolinones and aryloxy phenoxy propionate not only provide excellent selectivity but are used at extremely low dosage(Heath, 1995).

The long persistence of many herbicides in freshwater suggests that they are capable of producing adverse effects on freshwater organisms such as fish. Dalapon persist in water for 2 to 3 days, paraquat and diquat persist more than dalapon, and 2,4-D amine salt persist for 4 to 6 weeks; chlorthiamid breaks down into dichlobenil that stays for three months in water. On the other hand, terbutryne and diuron persist for more than three months in the water. These periods of time in the water show that most herbicides will cause serious adverse effects in the populations of freshwater organisms. (Newbold, 1975).

Vestaline®(Pendimethalin) is one of the only current use pesticides to be placed on the United States Environmental Protection Agency's Persistent, Bioaccumulatory, and Toxic list as part of the Toxic Release Inventory (USEPA, 1997).Vestaline®(Pendimethalin) has the following Properties: Chemical formula: $C_{13}H_{19}N_3O_4$; Molar mass: 281.31 g/mol; Density: 1.17 g/cm3; Melting point: 47 to 58 °C (117 to 1331 K); Boiling point: 330°C (626F); Solubility in water: 0.275ppm; Chemical Name: n-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitrobenzenamine.

The synthesis of pendimethalin (Dinitroaniline) can result in the synthesis of nitrosamines, a harmful and undesirable byproduct. Nitrosamines are highly reactive chemical species. They are metabolized to acid (HNO_2), which acts in biological systems by deaminating molecules. They have been implicated as carcinogens by removing amino groups from DNA chromosome nucleotide bases (i.e. cytosine, adenine, tyrosine, and guanine). They also

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can act as toxic alkylating agents (Doichevaet al., 2009). Pendimethalin is classified as persistent bioaccumulative toxic (PBT) and a group C carcinogen "possible human carcinogen" (USEPA, 1997). *Clarias gariepinus*is a rich source of animal protein, it is a highly palatable fish and its preferred for culture because of its high growth rate, hardy nature and prolific breeding in confined water. It is found in all the fresh waters sources in Nigeria and the most cultured fish in the country. This study is aimed at assessing the response of *Clarias gariepinus* exposed to different concentrations of Vestaline® (Pendimethalin) herbicide.

METHODOLOGY

Study Area

The study was carried out in the Department of Fisheries and Aquaculture, University of Agriculture Makurdi, Benue State. (Figureure1). Makurdi is locatedLatitude 7° 44' 1.50" N and Longitude: 8° 31' 17.00" E and it is situated at elevation 104 meters above sea level.

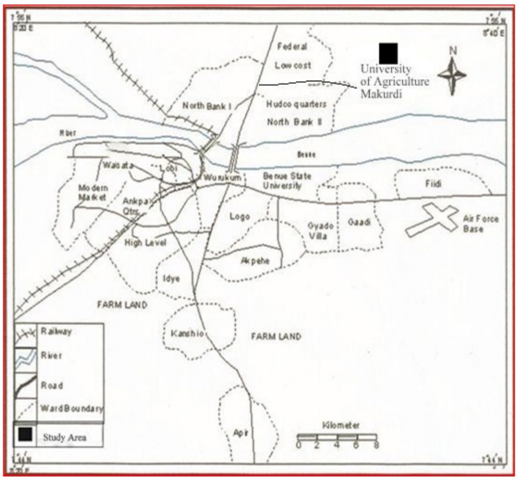

Figure 1: Map of Makurdi showing the study area.
Source: Wikipedia.com (2015)

Sample Collection

A total number of 450 (fourhundred and fifty)healthy and active juveniles of African catfish *Clarias gariepinus* with mean weight of 27.97±0.03g were collected from University of Agriculture Makurdi fish farm for the study. The fish were transported in plastic containers that were well aerated to the Department of Fisheries and Aquaculture, University of Agriculture, Makurdi. The fish were acclimatized in the department hatchery for two weeks during which they were fed twice daily with commercial floating feeds (coppens) at 5% of their body weight. Unconsumed feeds were removed and water replenished twice a week. Feeding started 24 hours after the arrival and stopped 24 hours before the commencement of the experiment as recommended by (Fafioyeet al., 2004).The herbicide Vestaline® (Pendimethalin) manufactured by Aidereim Limited UK was obtained from Franken technologies Agrochemical shop behind Benue state library Wurukum, Makurdi. Benue state

Preparation of the Concentrations Used For the Study

The stock solution of Vestaline® contains 330g/L of Pendimethalin and the volume used for the acute bioassay were 2.00mL,5.00mL,7.00mL,9.00mL and 11.00mL which were converted to 33mg/l 82.5mg/l,115.5mg/l,148.5mg/l and 181.5mg/l respectively using the formula C1V1=C2V2.(Odo, 2019) About 20 litres of dechlorinated water was poured into each tanks while 2.00, 5.00, 7.00, 9.00 and 11.00ml of water were removed and replaced with the same volume of Vestaline from the stock solution in each of the respective tanks to make it up to 20 litres mark.

Preliminary Test

After the acclimation period of 14 days, preliminary test was carried out to determine the suitable concentrations of the herbicide to be used for the study. This was done by introducing five different concentrations into five tanks (using adjustable micropipette) containing 20 litres of dechlorinated water. Ten fish per concentration of toxicant were used with replicates each for 96 hours. This procedure was repeated several times. Taking range from the concentrations at which 0% and 100% mortality was recorded (33.00mg/l, 82.5mg/l,115.5mg/l,148.5mg/l and 181.5mg/l) were gotten and used for this research.

AcuteToxicity Test

The methods of acute toxicity tests as described by Cengiz *et al*, (2001), Adeyemo (2005), Ayoola (2008) andAzua and Akaahan. (2017) was employed.
The concentrations of Vestaline® (Pendimethalin). (33mg/L,82.5mg/L,115.5mg/L,148.5mg/Land 181.5mg/L) obtained from the preliminary tests were dispensed using a micropipette into 20 litres of water contained in each test tank in triplicate.Control (0.00mg/L) was also setup.Ten (10) healthy juveniles of C. gariepinusfish were selected randomly and stocked in each tank. The juveniles were not fed 24 hours prior to the commencement of the study. The exposure period lasted for 96 hours with behavioral changes observed by visual observation (Ayubaet al., 2012; Abubakar and Abdulsalami, 2013).During this period fish mortality was observed and recorded every 12, 24, 48, 72 and 96 hours). The (96hr LC50)was estimated by probit analysis and slope function as recommended by (Ayoola, 2008.

Physicochemical Parameters of the Test Solution

Physicochemical parameters determined were Temperature, pH, Total dissolved solids (TDS), Electrical conductivity (EC) andDissolved oxygen (DO) using methods described by (Odo, 2019)

Data Analysis

Datawere analyzed using Minitab 17 for analysis of variance (ANOVA) and Regression at 0.05% level.

RESULTS

Behavioural Behavioral changes (Aggression, Excessive mucus secretion, Erratic swimming, Air gulping, and Discoloration) observed on Clarias gariepinus juvenile sexposed to Vestaline (Pendimethalin) herbicide all increased with increase in concentrations (Table 1).

Mortality was observed in all the tanks during the time of exposure (96hrs) except in the control tanks (Table 2). Mortality increased with increase in concentration with treatment 6 (181.5 mg/L) having the highest mortality. Figureure 2 showed that Mortality was first observed at 12 hours in 148.5mg/L and 181.5mg/L of Vestaline®herbicide to be 6 and 7 respectively while the other treatments had no mortality but as time progresses death was inevitable in all treatments accept in control (0.00). The result obtained from this research work showed the 96 hour LC50to be 113.56mg/L; this was determined by plotting the graph of probit kill values against log of concentration and finding the values of 50% probit kill as against the antilog value on log concentration value (Figure 3).

The regression relationship between concentrations of Vestaline® and mortality rate was calculated to be, R2 = 0.9719 and the upper and lower limits having values of 102.08mg/L and 124.96 mg/L respectively.

The result of the physico-chemical parameters of the water used in the acute bioassay (Table3)Showed that all the parameters determined increased significantly ($P<0.05$) with increase in concentration except Dissolved oxygen which decreased with increase in concentration. On the other hand there was no significant difference ($P>0.05$) between the control and the other treatments for temperature.

DISCUSSION

The present study investigated the acute toxicity of

Table 1: Behavioural Changes of *Clarias Gariepinus* juveniles Exposed to Tovestaline (Pendimethalin) Herbicide for 96 Hours.

Behavioural changes	**Concentration (mg/L)**					
	0.00	**33.00**	**82.50**	**115.50**	**148.50**	**181.50**
Aggression	-	-	-	++	++	++
Excessive mucus secretion	-	-	++	++	++	+++
Erratic swimming	-	+	++	++	+++	+++
Air gulping	-	++	++	+++	+++	+++
Discoloration	-	-	++	++	+++	+++

KEYS: None = (-)Weak = (++) Strong = (+++)

Table 2: Mortality Rates of *Clarias Gariepinus* juveniles Exposed to Acute Concentrations of Vestaline (Pendimethalin) Herbicide For 96 Hours.

Concentration (mg/l)	**No. of exposed Fish**	**Mortality Rate**	**% Mortality**	**Log of Conc**	**Probit kill**
0.00	30	0.00	0.00	0.00	0.00
33.0	30	1	3.33	1.52	3.12
82.5	30	8	26.67	1.92	4.36
115.5	30	16	53.33	2.06	5.08
148.5	30	24	80.00	2.17	5.71
181.5	30	29	96.67	2.26	6.28

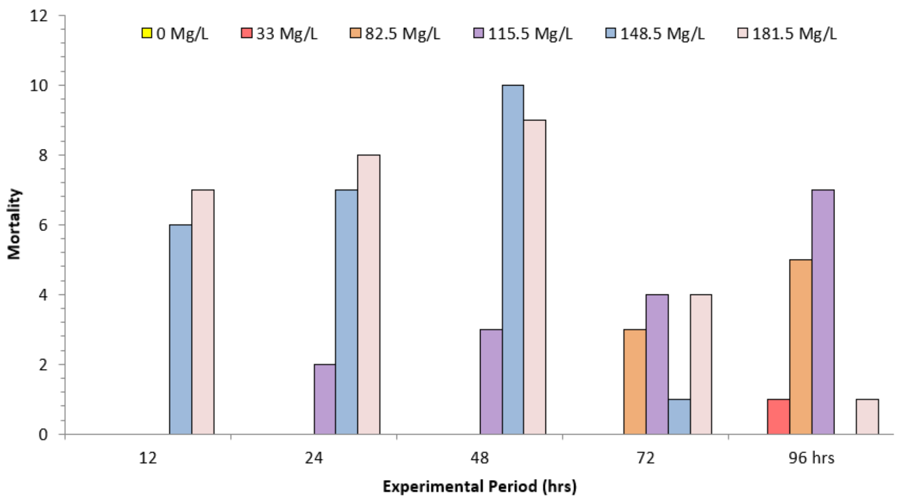

Figure 2: Mortality Rates of Clariasgariepinusjuveniles Exposed to Acute Concentrations of Vestaline (Pendimethalin) Herbicide For 96 Hours.

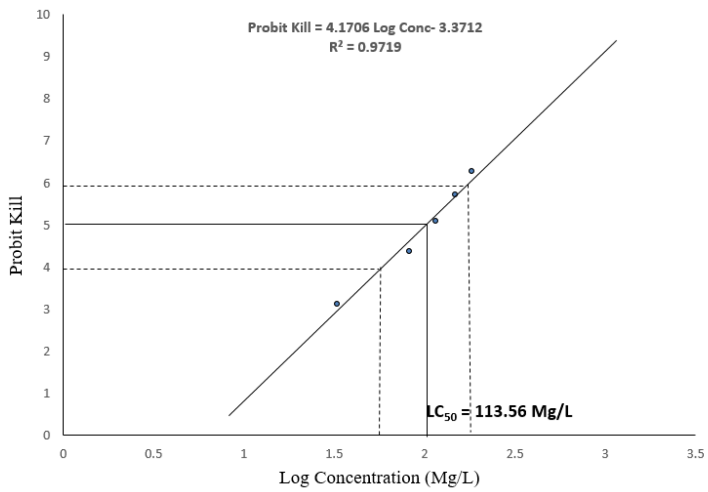

Figure 3: Linear Relationship Between Probit Kill and Log Concentration of clariasgariepinus Juveniles Exposed to Various Acute Concentrations of Vestaline (Pendimethalin) Herbicide for 96 Hours.

Table 3: Mean Physico-Chemical Parameters of the Water During Exposure of the Juveniles of *Clarias Gariepinus*to Acute acute Concentrationsof Vestaline (Pendimethalin) Herbicide for 96 Hours.

Water Quality parameter					
Concentration(mg/L)	Temperature(°C)	pH	Total Dissolved Solids(mg/L)	Electrical Conductivity (µS/cm)	Dissolved Oxygen(mg/L)
0.00	26.25±0.25	7.80±0.10c	98.65±0.05f	197.05±0.65f	5.75±0.15a
33 .00	26.60±0.30	8.35±0.05c	125.85±0.55e	251.30±0.70e	4.70±0.10b
82.50	26.20±0.10	9.50±0.20b	129.65±0.05d	259.60±0.20d	3.90±0.10c
115.50	27.50±0.50	9.60±0.20b	132.85±0.55c	264.65±0.05c	3.50±0.10d
148.50	26.25±0.05	10.65±0.55a	138.70±0.50b	273.25±0.15b	3.35±0.15d
181.50	26.60±0.30	11.50±0.00a	141.25±0.65a	284.40±0.60a	2.85±0.05e
P-Value	0.71ns	0.02	0.03	0.01	0.01

Means on the Same Column with Different Superscript are Statistically Significant (P<0.05) Ns = Not Significant

commercially soldVestaline® (Pendimethalin) herbicide on the African catfish *Clarias gariepinus*. Behavioural studies have been reported with fish exposure to environmental contamination and pollutants associated with exposure of fish to environmental contamination and pollutants have been reported (Ahmad, 2011). There were behavioral changes in the activities of Clariasgarienpinustreated with different acute concentrations of Vestaline herbicide compared to the control. Among these changes were the periphery of dorsal, caudal fins and belly coloured in yellow from the herbicide, hyperactivity, increased erratic swimming, decreased equilibrium status, decreased fin movement and excessive mucus secretion, air gulping and fish settling at the bottom motionless with slow opercula movement before death.

This result agrees with Nabela*et al.* (2011) who observed similar behavioral alterations when Oreochromisniloticus were exposed toStomp (Pendimethalin) herbicide at different concentrations.The observed behavioral alterations in the studied herbicide are consistent with other previous reports on pesticides such as cypermethrin (Ansari *et al.*, 2011) profenofos (Pandey *et al.*, 2011), atrazine (Nwani*et al.*, 2011), diazinon (Ahmad, 2011), endosulfan (Yekeen and Falowe, 2011; Shao *et al.*, 2012), carbosulfan(Altinok*et al.*, 2012) and malathion Ahmad, 2012).The abnormal behavioral alterations in Vestaline herbicide-exposed fish may indicate disturbance in the internal physiology of the fish, which may be attributed to the neurotoxic property of the herbicide. Studies by Bull *et al.* (2007) indicated that Cyperdicot (pesticide) interferes with signalling at the neuromuscular junction, thereby disrupting the Ca2+ voltage-gated channels. This will result in conformational changes in membrane lipid and membrane fluidity, which further indicates a neuro-pharmacological effect of pesticides on the fish (Wilson *et al.*, 2003). According to Sarai *et al.* (2013), this is a further indication of a neuro pharmacological effect of pesticides on the fish (Wilson *et al.*, 2003). According to Sarai *et al.*, (2013), this would result in prolonged neuromuscular depolarisation, culminating in the observed uncoordinated and jerky movement that was noticed in Vestaline exposed fish. Or the behavioural alteration may occur as a result of nervous impairment due to blockage of nervous transmission among the system and various effecter sites as reported by Fryday*et al.*, (1996).

The stressful behaviour of respiratory impairment due to the toxic effect of Vestaline on the gills was similar with the reports of Omitoyin*et al.*, (2006) and Aguigwo (2002) who reported that pesticide impairs respiratory organs. The toxicity of Vestaline® herbicide to the freshwater fish Clariasgariepinuswas studied using static bioassay method. A regular trend was observed in the mortality rate during acute toxicity assay which increased with increased concentration. At the early stage (the first 6 hours) of the toxicant introduction, all the fishes survived the that is the first 6 hours) of the toxicant introduction, all the fishes survived the initial attack. This may be due to the protectiveadaptations and the hardy nature of C. gariepinus. After 12 h of exposure the fish displayed physiological malfunctions and subsequently death. These were more noticeable among some fish in the highest concentrations (148.5mg/Land 181.5mg/L). With progressive exposure 96 h, deaths became inevitable even at lower concentrations. This could be due to stress and cumulative impact of the herbicide. The result showed that the mortality of fish were dose-dependent. This observation is similar to the report of Azua and Akaahan, (2017). The acute toxicity results showed that higher concentrations Results of acute toxicity showed that higher concentrations of Vestaline (Pendimethalin) herbicide cause death. This can be seen in the relationship between concentrations of Vestaline and mortality with R2 value of 0.9719. The mortality recorded in the study is considered a consequence of stress induced on fish's immune system. the immune system of fish Thus, slow toxic progress and long continuance can result into chronic toxic response. Water quality parameters plays a great role in causing the toxicity by different pesticides that ultimately have harmful effect on diversity, abundance and dynamics of aquatic flora and fauna. The presence of pesticides in water has offensive taste, odour and colour to fish and aquatic plants even when they are present in low concentrations (De, 2003).

The results of physico-chemical parameters of water obtained during the exposure to the various concentrations showed decreased dissolved oxygen and increased in dissolved oxygen and increased Electrical conductivity (EC), Total Dissolved Solids(TDS) and pH while Temperature showed no significant difference. Although the level of parameters differed significantly with increase in concentration, they were still within acceptable limits for survival of aquatic organisms(Faioye*et al.* 2004). The reduction on oxygen concentrations can be due toincreased activities of the fish due to the herbicide which can reduce oxygen from the water and also in the presence of adverse environment, organisms intends to adjust itself for survival this could cause increase uptake of oxygen.

CONCLUCTION

The results of the acute toxicity showed that Vestaline (Pendimethalin) herbicidewas toxic to African catfish Clariasgariepinus, with LC50 value of 113.56mg/L. The mortality threshold indicates it is very toxic with increase concentration because the results of the regression analysis of the exposed concentrations of the toxicant to the mortality of the fish indicate a strong relationship between the concentrations and the mortality of Clariasgariepinuswith R2 value of 0.9719.

Sub-lethal concentrations of Vestaline (Pendimethalin) herbicide in the aquatic environment will not necessarily result in outright mortality of aquatic organisms but can bio accumulate over a period of time which may constitute potential health hazards not only to the aquatic organisms like fish but also on higher trophic level especially man.

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